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Supplementary Protocol

QIAprep&amp Viral RNA UM Kit Protocol for Analysis of Saliva and Gargle Samples

This supplementary protocol is for the detection of SARS-CoV-2 in saliva or gargle samples from human donors, using the QIAprep&amp Viral RNA UM Kit (cat. nos. 221413, 221415, and 221417) in combination with the SARS CoV-2 N1+N2 Assay Kit (cat. nos. 222015 and 222017), in epidemiological research. This supplementary protocol is intended for molecular biology application, not for molecular diagnostic use. For increased performance with saliva and gargle samples, we recommend the protocol version using of the QIAprep&amp Buffer AB (cat. nos. 221513, 221515, and 221517).

Further information

- *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**
- *QIAprep&amp Buffer AB Quick-Start Protocol*: **www.qiagen.com/HB-2887**
- *SARS-CoV-2 N1+N2 Assay Kit Quick-Start Protocol*: **www.qiagen.com/HB-2829**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Personal safety equipment
- A programmable real-time PCR thermocycler with at least three detection channels, compatible with the QIAprep&amp Viral RNA UM Kit (Table 1)
- Plastic PCR consumables compatible with the abovementioned thermocyclers
- Calibrated micropipettes for volumes ranging from 2 µl to 50 µl and tips or an automated liquid handler

In place of QIAGEN's SARS CoV-2 N1+N2 Assay Kit (cat. nos. 222015 and 222017), it is possible to use another probe-based assay for the detection of one or more targets from RNA viruses, compatible with the QIAprep&amp Viral RNA UM Kit (Table 1). We suggest to use FAM or another equivalent dye that can be detected simultaneously with the Internal Controls Assays contained in this kit in a multiplex PCR. The assay is referred to as primer–probe mix in the reaction setup table (Table 2).

Notes before starting

- The Viral RNA UM Prep Buffer prepares the samples for the detection step but is not a virus inactivation solution, and QIAGEN cannot guarantee that the heat treatment step will inactivate 100% of viral particles. The inactivation of virus needs to be verified and validated by users.
- The RT-qPCR protocol uses TaqMan probes in a multiplex reaction that works with any real-time cyclers. For fluorescence normalization, ROX dye might be required (refer to the kit handbook for instructions).

  Important: Always start with the cycling conditions specified in this protocol.
- The PCR section of the RT-qPCR protocol must start with an initial incubation step of 2 minutes at 95°C to activate the DNA Polymerase.
- For viral targets, it is recommended to prepare a 20x primer–probe mix containing target-specific primers and probes. We recommend to use a final concentration of 0.8 µM primers (forward/reverse) and 0.25 µM probe in the reaction.
- The RNA IC Template + Assay is an inhibition control using a synthetic RNA template. It is a 200 bp IC template detected in the red channel on the Rotor-Gene® Q or in the Cy5® channel on other real-time PCR instruments.
- The Human Sampling IC Assay (Sampling Control) is intended to report that the primary sample tube contains intact human genetic material. For this purpose, two different human targets are both detected in the yellow channel on the Rotor-Gene Q or in the VIC®/HEX™ dye channel on other real-time PCR instruments. The pre-mixed formulation (20x) contains forward and reverse primers and TaqMan probes.
- The SARS-CoV-2 N1+N2 Assay Kit contains tubes with a mixture of four primers and two probes purified by HPLC, at a 20x concentration. The four primers are based on the CDC design (**https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html**), targeting regions N1 and N2 of the viral genome. The two probes are coupled with FAM™ as a reporter dye and use ZEN™ quenchers for enhanced sensitivity
- Before use, thaw the Viral RNA UM Prep Buffer, Viral RNA Master Mix, RNA IC Template + Assay, Human Sampling IC Assay, ROX Reference Dye (if required), and RNase-Free Water. Mix the individual solutions.

Table 1. Overview table of the multiplex RT-PCR assay

Assay	Targets	Dye/color channel	Supply
SARS-CoV-2 N1+N2 Assay	N1 and N2 genes	FAM/Green (the two targets detected in the same channel)	SARS-CoV-2 N1+N2 Assay Kit
Inhibition control	Synthetic IVT	Similar as Cy5/Red	QIAprep&amp Viral RNA Kit, optional use but recommended
Sampling control	Human B2M and RNase P genes	HEX/Yellow (the two targets detected in same channel)	QIAprep&amp Viral RNA Kit, optional use but recommended
Passive reference dye	To be used only for real-time cyclers that need this reference dye	ROX/Orange	QIAprep&amp Viral RNA Kit, optional use but recommended

Protocols for Saliva and Gargle Samples

The QIAprep&amp Viral RNA UM Kit is an innovative liquid-based method optimized for the preparation and detection of viral RNA targets from saliva and swab samples such as nasal, nasopharyngeal, or oropharyngeal swabs that are stored in non-fixation transport media such as UTM, VTM, PBS, ESwabs®, Virocult™, or 0.9% NaCl.

This supplementary protocol describes two protocols for the analysis of pure/neat saliva and gargle samples in 0.9% NaCl with QIAprep&amp Viral RNA UM kits. These two protocols encompass different sample pre-treatments: one protocol with the QIAprep&amp Buffer AB and the other without it (Figure 1).

The QIAprep&amp Buffer AB is designed to further increase the sensitivity of the QIAprep&amp Viral RNA workflow when using sample types such as pure/neat saliva and gargle samples, or other sample types requiring pre-treatment. Ct values obtained with the QIAprep&amp Buffer AB are up to 2Ct earlier compared to the control workflow; see data on the product page (**www.qiagen.com/qiaprepandamp-resources**).

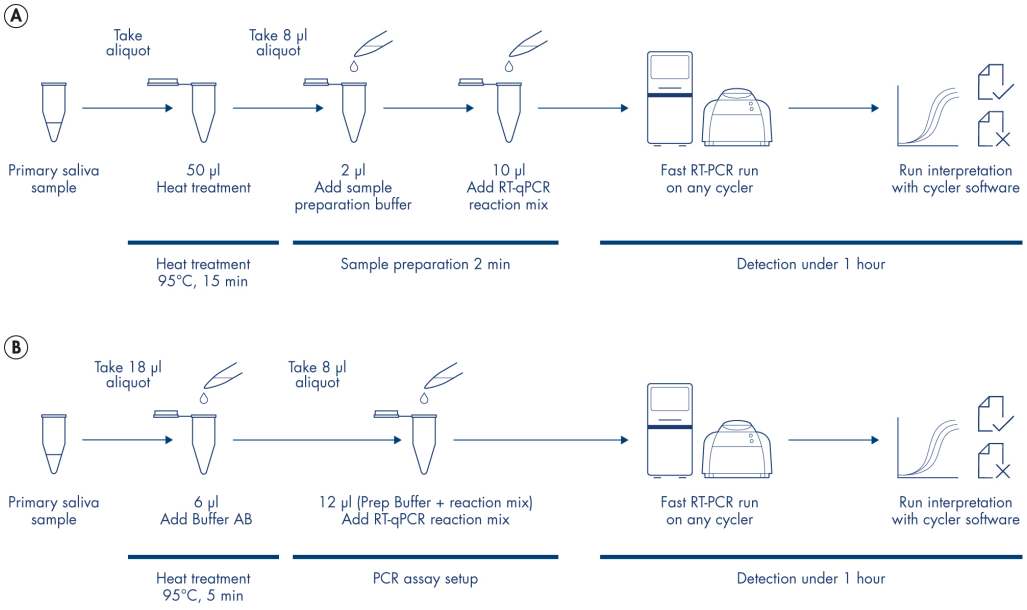

Figure 1. Overview of the two QIAprep&amp Viral RNA UM Kit protocols for saliva and gargle samples. (A) Workflow without the use of the QIAprep&amp Buffer AB and (B) workflow with the QIAprep&amp Buffer AB for greater sensitivity.

Procedure Without the Use of QIAprep&amp Buffer AB

Heat pre-treatment of samples

1. Saliva/gargle sample pre-treatment:
    1a. Vortex saliva or gargle samples vigorously. Either the entire primary sample or an aliquot of 50 µl can be submitted for heat treatment. Ensure the complete sample volume is appropriately heated, especially for volumes of samples exceeding 100 µl. Bigger volumes would require longer incubation times
    1b. Incubate at 95°C for 15 min.
    1c. Centrifuge the plate/tube briefly.

    Note: Saliva samples can have high viscosity. Heating the primary sample to 80°C for 10 min can lower viscosity of the sample and facilitate pipetting of saliva samples. Heating the primary sample to 80°C for 10 min does not replace the heating step at 95°C for 15 min in step 1b.

RT-PCR setup

2. Dispense 2 µl of Viral RNA UM Prep Buffer into a new PCR tube or well of a PCR plate.
3. Gently mix by pipetting up and down the heat pre-treated sample prepared in step 1c and transfer 8 µl to the individual PCR tube or wells containing the Viral RNA UM Prep Buffer. Mix by pipetting up and down at least two times.
4. Incubate at room temperature for 2 min.

    Note: Incubation time starts after adding the last sample to the Viral RNA UM Prep Buffer.
5. Prepare a reaction mix according to Table 2 and mix thoroughly.

Table 2. Reaction mix setup

Component	96/384-well block	Final concentration
Viral RNA UM Master Mix, 4x*	5 µl	1x
SARS-CoV-2 Assay, 20x	1 µl	1x
RNA IC Template + Assay, 10x*	2 µl	1x
Human Sampling IC Assay, 20x*	1 µl	1x
ROX Reference Dye (ABI instruments only)*	1 µl/0.1 µl†	1x
RNase-Free Water*	Fill up to 10 µl	-
Prepared sample (added at step 1)	10 µl	-
Total reaction volume	**20 µl**	**–**

* Kit component of QIAprep&amp Viral RNA UM Kit (cat. nos. 221413, 221415, and 221417). Refer to the *QIAprep&amp Viral RNA UM Handbook* for safe and proper use.

† To be used as a 20x concentrate for high ROX cyclers (i.e., ABI PRISM 7000, Applied Biosystems® 7300 and 7900, and StepOne® Real-Time PCR Systems) and as a 200x concentrate for low ROX dye cyclers (i.e., Applied Biosystems 7500, ViiA7™, and QuantStudio® Real-Time PCR Systems).

6. Add 10 µl of the reaction mix prepared in step 5 to the PCR tubes or wells prepared in step 4.
7. **Important consideration**:
    7a. Seal the plate/tube thoroughly to prevent cross-contamination. In case an adhesive film is used, make sure to apply pressure uniformly across the entire plate, to obtain a tight seal across individual wells.
    7b. Mix gently by vortexing for 10–30 s at medium speed. Place the plate in different positions while vortexing, to ensure an equal contact with the vortex platform.
    7c. Centrifuge the plate/tube briefly to collect liquid at the bottom of the plate/tube.
    7d. Immediately proceed to step 8. The complete reaction can be stored up to 1 h at room temperature or for a longer period, frozen at –30 to –15°C.
8. Place the tubes or plates in the real-time cycler and perform cycling according to the below conditions (Table 3).

    Program the real-time cycler before reaction setup according to Table 3.

    Note: Data acquisition should be performed during the annealing/extension step.

Table 3. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step	10 min	50°C	Maximal/fast mode
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling (40 cycles)			
Denaturation	5 s	95°C	Maximal/fast mode
Combined annealing/extension	30 s	58°C*	Maximal/fast mode

* Annealing temperatures can be adapted between 55–62°C depending on primer/probe set used. For further details on cycling conditions, primer/probe concentrations, and annealing temperature, visit the product page (**www.qiagen.com/qiaprepandamp-resources**).

9. For results interpretation, refer to the table "Possible outcome" in the *QIAprep&amp Viral RNA UM Kit Handbook:* **www.qiagen.com/HB-2830**.

Procedure with the Use of QIAprep&amp Buffer AB

Heat pre-treatment of samples with QIAprep&amp Buffer AB

1. Before use, prepare the QIAprep&amp Buffer AB according to Table 4 and mix thoroughly.
2. Pre-dispense 6 µl of Buffer AB into a PCR tube or the well of a PCR plate.
3. Vortex saliva or gargle samples vigorously. Add 18 µl of sample to the tube or well containing the Buffer AB. Mix by pipetting up and down at least twice.

    Note: Saliva samples can have high viscosity. Heating the primary sample to 80°C for 10 min can lower viscosity of the sample and facilitate pipetting of saliva samples. Heating the primary sample to 80°C for 10 min does not replace the heating step at 95°C for 5 min in step 4.

4. Seal the tube/plate and incubate for 5 min at 95°C.
5. Briefly centrifuge the tubes. Gently mix by pipetting up and down at least twice, and transfer 8 µl of heat pre-treated sample into a new PCR tube or well and proceed with step 6 below.

Table 4. QIAprep&amp Buffer AB setup

Component	1 rxn	Final concentration
QIApre&amp Buffer A	3.75 µl	1x
QIApre&amp Buffer B*	2.25 µl	1x
Total reaction volume	**6 µl**	**–**

* contains Proteinase K

PCR setup

6. Prepare a reaction mix according to Table 5 and mix thoroughly.
7. Add 12 µl of the reaction mix prepared in step 6 to the 8 µl of sample prepared in step 5. Mix by pipetting up and down at least twice. The complete reaction can be stored up to 1 h at room temperature or for a longer period, frozen at –30 to –15°C.

Table 5. Reaction mix setup

Component	96/384-well block	Final concentration
Viral RNA Master Mix, 4x*	5 µl	1x
SARS-CoV-2 Assay, 20x	1 µl	1x
RNA IC Template + Assay, 10x*	2 µl	1x
Human Sampling IC Assay, 20x*	1 µl	1x
ROX Reference Dye (ABI instruments only)*	1 µl/0.1 µl†	1x
Viral RNA UM Prep Buffer*	2 µl	1x
RNase-Free Water*	Fill up to 12 µl	-
Prepared sample (after step 5)	8 µl	-
Total reaction volume	**20 µl**	**–**

* Kit component of QIAprep&amp Viral RNA UM Kit (cat. Nos. 221413, 221415 and 221417). Refer to the *QIAprep&amp Viral RNA UM Handbook* for safe and proper use.

† To be used as a 20x concentrate for high-ROX dye cyclers (i.e., ABI PRISM® 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for low ROX-dye cyclers (i.e., Applied Biosystems 7500, ViiA7, and QuantStudio Real-Time PCR Systems).

8. **Important consideration**:
   - 8a. Seal the plate/tube thoroughly to prevent cross-contamination. In case an adhesive film is used, make sure to apply pressure uniformly across the entire plate, to obtain a tight seal across individual wells.
   - 8b. Mix gently by vortexing for 10–30 s with medium pressure. Place the plate in different positions while vortexing, to ensure an equal contact with the vortex platform.
   - 8c. Centrifuge the plate/tube briefly to collect liquid at the bottom of the plate/tube.

9. Program the real-time cycler according to Table 6.

Note: Data acquisition should be performed during the annealing/extension step.

Table 6. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step	10 min	50°C	Maximal/fast mode
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling (40 cycles)			
Denaturation	5 s	95°C	Maximal/fast mode
Combined annealing/extension	30 s	58°C*	Maximal/fast mode

* Annealing temperatures can be adapted between 55–62°C depending on primer/probe set used. For further details on cycling conditions, primer/probe concentrations, and annealing temperature, visit the product page (**www.qiagen.com/qiaprepandamp-resources**).

10. Place the tubes or plates in the real-time cycler and start the cycling program.
11. For results interpretation, refer to the table "Possible outcome" in the *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**.

Ordering Information

Product	Contents	Cat. no.
QIAprep&amp Viral RNA UM Kit (100)	For 100 x 20 µl reactions: 0.24 ml Viral RNA UM Prep Buffer; 0.5 ml Viral RNA Master Mix, 4x; 0.2 ml RNA IC Template + Assay; 0.1 ml Human Sampling IC Assay; 1 ml QN ROX; 1 x 1.9 ml RNase-Free Water	221413
QIAprep&amp Viral RNA UM Kit (600)	For 600 x 20 µl reactions: 1.2 ml Viral RNA UM Prep Buffer; 2 x 1.5 ml Viral RNA Master Mix, 4x; 1.2 ml RNA IC Template + Assay; 0.6 ml Human Sampling IC Assay; 1 ml QN ROX; 2 x 1.9 ml RNase-Free Water	221415
QIAprep&amp Viral RNA UM Kit (2400)	For 2400 x 20 µl reactions: 4 x1.2 ml Viral RNA UM Prep Buffer; 8 x 1.5 ml Viral RNA Master Mix, 4x; 4 x1.2 ml RNA IC Template + Assay; 4x0.6 ml Human Sampling IC Assay, 4 x 1 ml QN ROX; 8 x 1.9 ml RNase-Free Water	221417
SARS-CoV-2 N1+N2 Assay Kit (600)	For 600 x 20 µl reactions: 1x 600 µl SARS-CoV-2 N1+N2 assay, 20x concentrate	222015
SARS-CoV-2 N1+N2 Assay Kit (2400)	For 2400 x 20 µl reactions: 4x 600 µl SARS-CoV-2 N1+N2 assay, 20x concentrate	222017

Product	Contents	Cat. no.
QIAprep&amp Buffer AB (0.7 ml)	Buffer for the pre-treatment of 100 samples. For use with QIAprep&amp Viral RNA kits	221513
QIAprep&amp Buffer AB (4.1 ml)	Buffer for the pre-treatment of 600 samples. For use with QIAprep&amp Viral RNA kits	221515
QIAprep&amp Buffer AB (16.4 ml)	Buffer for the pre-treatment of 2400 samples. For use with QIAprep&amp Viral RNA kits	221517

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Document Revision History

Date	Changes
04/2021	Initial release
01/2022	Added the catalog number for the new kit size (100 rxn; cat. no. 221413). Added catalog number for the new size of the QIAprep&amp Buffer AB (cat. no. 221513).

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